

Long-Term Effects of Hypophysectomy on the Growth and Calcification of Otoliths and Scales in the Goldfish, *Carassius auratus*

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ABSTRACT—Goldfish (*Carassius auratus*) were given tetracycline for time-marking, followed by hypophysectomy. They were kept at 0.25% NaCl-tap water for 32 weeks and then sacrificed to examine long-term effects of hypophysectomy on the accretive growth and calcification of otoliths and scales. Plasma was also analysed for calcium and sodium concentrations.

Hypophysectomy resulted in cessation of somatic growth in length. Hypercalcemia and hyponatremia were induced by hypophysectomy. Tetracycline-induced bands indicated that scale growth almost ceased after operation, while otoliths continued to grow at a rate reduced to approximately half. The marginal osteoid of scales was poorly developed in hypophysectomized fish. X-ray energy spectra revealed no difference in elemental constituents of the newly formed regions of these tissues between the experimental and control groups: major elements were phosphorus and/or calcium, suggesting that these regions are fully calcified. These results were discussed with the allometric growth of otoliths to somatic growth.

INTRODUCTION

Teleost otoliths and scales are mainly composed of calcium salts, and are used for age determination. They grow by the deposition of calcium carbonate or phosphate on the non-collagenous or collagenous matrix. Their growth has been considered to be isometric to somatic growth, at least before the somatic growth becomes asymptotic. However, Secor and Dean [1] have recently indicated the presence of two components in otolith growth: one is an endogenously defined incremental growth, which can occur during periods of decreased or arrested somatic growth, and the other is a continuous growth within a day in some proportion to daily somatic growth. They have proposed a daily increment packing (DIP) model for such a mode of otolith growth. The DIP model suggests that somatic and otolith growth is not necessarily isometric in detail.

Somatic growth is under endocrine control, and

it has repeatedly been reported that the removal of the pituitary gland results in cessation of fish growth in length [2–4]. Pickford [2] examined otoliths and scales from hypophysectomized killifish and found no new circulus formation in the scales and no new deposition on the otoliths. Her findings came from a morphological comparison of the outermost margin of these tissue between hypophysectomized and control fish. Therefore, it remains uncertain how, quantitatively, hypophysectomy affects the growth rate of otoliths and scales.

The present study was undertaken to examine quantitatively long-term effects of hypophysectomy on the growth and calcification of otoliths and scales using tetracycline-labeled goldfish.

MATERIALS AND METHODS

The goldfish, *Carassius auratus*, weighing approximately 21.7 g were obtained from a commercial dealer and acclimated to experimental conditions for at least 2 weeks before use. They were maintained at $23 \pm 1^\circ\text{C}$ and fed carp pellets once a

day throughout the acclimation and experimental periods.

Twenty fish were given a single intraperitoneal injection of tetracycline (Takeda Chemical Ind.) at a dose of 0.1 mg/g body weight and allowed to recover for 4 days. Then, they were randomly divided into 2 groups and one group was hypophysectomized by the opercular approach technique [5]. The other group was subjected to drilling of the prootic bone only, and used as sham-operated control.

Hypophysectomized and sham-operated fish were separately placed into one of the two compartments halved by a wire-netting in a 120 l tank containing aerated and filtrated 0.25% NaCl tap water (Na conc. 43.1 mM and Ca conc. 0.2 mM). They were taken out for measurement of body size twice a month and returned to the other compartment each time. This replacement made their environmental conditions as even as possible. No individual discrimination was made. The dividing netting was removed after about 3 months and both groups were maintained in the same compartment from then on. Nevertheless, it was easy to distinguish the experimental fish from the control, because the red body color had faded to almost white in the hypophysectomized fish. They were sacrificed 32 weeks after operation.

Blood was collected from the caudal vessels by cutting the tail of the fish and draining it into heparinized (as Li salt) capillary tubes. After centrifugation, the separated plasma was stored at -40°C for 1 day and analysed for calcium and sodium concentrations by emission spectrophotometry (Hitachi, 518).

Somatic growth was expressed as a relative growth rate (RGR) after Ricker [6].

$$\text{RGR} = \frac{B-A}{T-t} \times \frac{1000}{A}$$

where A and B represent initial and final sizes, respectively, and T-t is the change in time (32 weeks). RGR in scales and otoliths was calculated in the same manner.

After blood collection, 6 scales were removed with forceps from the antero-dorsal part of each fish. Special attention was paid not to include regenerating scales. They were carefully rinsed

several times with distilled water and examined for tetracycline-induced fluorescence under ultra-violet illumination (Nikon, EFD). The distance of accretive growth during the experiment was measured with a calibrated eye-piece to the nearest μm in the anterior part of scales and RGR was calculated. After measurement, scales were stained with 1% silver nitrate [7] for calcium detection.

Otoliths (asterisci and lapilli) were dissected, rinsed with distilled water and dried. After measurements of weight and length in the dorso-ventral axis, asterisci were embedded within a mass of methacrylate of suitable hardness and ground down to approximately 100 μm in thickness so that the polished plane was parallel to the axis across the center. Ground otoliths were subjected to microscopic examination for fluorescence. The distance of accretive growth was measured in the dorsal and ventral directions. Only weight and length in the antero-posterior axis were measured in lapilli.

Student's t-test for unpaired observations was applied to assess statistical significance of differences between mean values. Significance was accepted at a P-value of <0.05 .

The degree of calcification of the newly formed regions of scales and otoliths was estimated by X-ray microanalyses. Scales and ground otoliths were ultrasonically cleaned in xylene and/or distilled water and mounted on a carbon holder with a carbon tape. After being coated with carbon, they were examined for elemental constituents at 20 kV by spot analyses using a Horiba EMAX-2000 energy-dispersive X-ray microanalysis system. Spectrum collection time was 100 sec.

RESULTS

Hypophysectomy resulted in a marked reduction in somatic growth (Table 1). Relative growth in weight gain was reduced by 70% in hypophysectomized fish compared to the sham-operated control. No increase in length was found in the experimental fish in spite of their active feeding.

Plasma sodium and calcium were significantly affected by hypophysectomy (Table 2). Although fish were maintained in 0.25% NaCl throughout the course of the experiment, sodium concentra-

TABLE 1. Somatic growth in hypophysectomized and sham-operated goldfish

	Sham-operated	Hypophysectomized
Body weight (g)		
Initial	21.0±1.0 (8)*	22.4±1.0 (9)
Final	33.6±3.0 (7)	26.2±1.6 (8)
RGR**	18.8	5.3
Standard length (cm)		
Initial	8.3±0.1 (8)	8.4±0.2 (9)
Final	9.3±0.3 (7)	8.3±0.1 (8)
RGR	3.77	-0.38

* Mean±SE (No. of fish examined).

** Relative growth rate.

TABLE 2. Plasma calcium and sodium concentrations (mEq/l) in hypophysectomized and sham-operated goldfish

	Sham-operated	Hypophysectomized	Significance
Ca	4.7±0.1 (7)*	5.2±0.2 (6)	P<0.05
Na	135.2±1.1 (7)	129.9±0.7 (6)	P<0.01

* Mean±SE (No. of fish examined).

TABLE 3. Otolith sizes in hypophysectomized and sham-operated goldfish

	Sham-operated	Hypophysectomized	Significance
Length (μm)			
Asteriscus	2988±64 (10)*	2605±22 (12)	P<0.001
Lapillus	1957±31 (12)	1879±22 (12)	
Weight (mg)			
Asteriscus	7.9±0.3 (10)	6.7±0.3 (12)	P<0.01
Lapillus**	8.0±0.4 (6)	6.4±0.2 (6)	P<0.01

* Mean±SE (No. of otoliths examined).

** A pair of lapilli were pooled for weighing.

tions decreased in hypophysectomized fish ($p<0.01$). On the other hand, hypophysectomy resulted in an increase in plasma calcium concentrations at the end of the experiment ($p<0.05$).

Measurements of whole otoliths showed that otolith growth was inhibited by hypophysectomy (Table 3). Asterisci of hypophysectomized fish gained significantly less ($p<0.01$), 13% and 15% less in length and weight respectively, than those of the control, while a significant ($p<0.01$) effect of hypophysectomy on lapillus growth was found

only in weight, showing a 20%-reduced gain. Tetracycline-induced bands visually confirmed the inhibitory effects of hypophysectomy on otolith growth in length (Fig. 1). Otolith growth toward the dorso-ventral directions is presented as a relative growth rate (Table 4). Hypophysectomy reduced the rates by half in both directions ($p<0.001$). The pattern of X-ray energy spectra was almost the same between the experimental and control otoliths. The heavy presence of calcium with a trace of phosphorus was confirmed in the

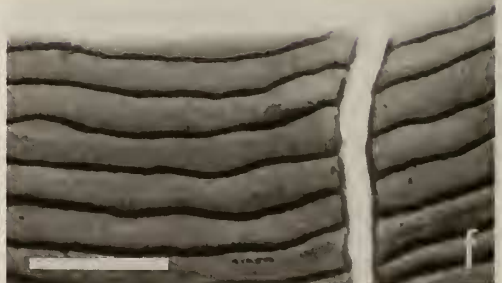
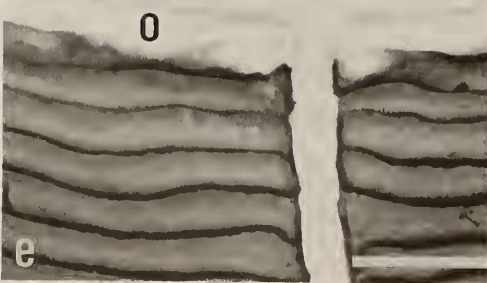
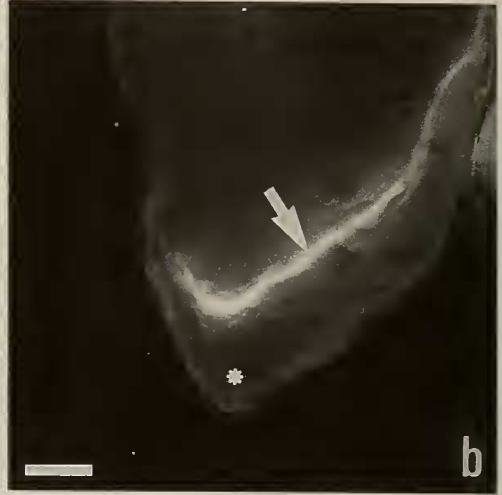
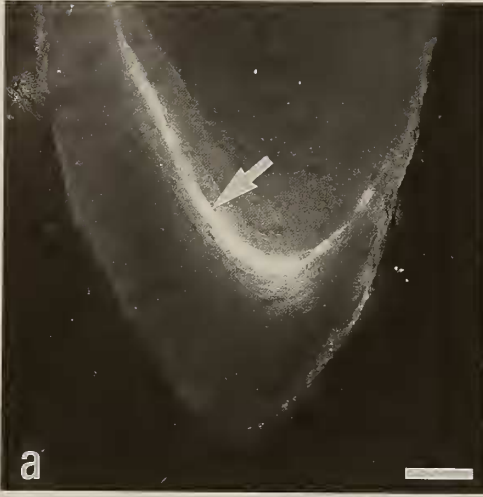


TABLE 4. Relative growth rates in otoliths and scales in hypophysectomized and sham-operated goldfish

	Sham-operated	Hypophysectomized	Significance
Otolith (asteriscus)			
Dorsal	5.91±0.38 (10)*	3.05±0.19 (14)	P<0.001
Ventral	4.93±0.33 (10)	2.44±0.20 (14)	P<0.001
Scale	6.43±0.76 (18)	0.62±0.06 (20)	P<0.001

* Mean±SE (No. of samples examined).

newly formed region following hypophysectomy (Fig. 2). Therefore this region should be calcified.

Scale growth was markedly reduced by hypophysectomy. Only 1 or 2 ridges were added to the hypophysectomized fish scales during the experiment, while 16–30 ridges were newly formed in the control (Fig. 1). Relative growth rates showed that hypophysectomy reduced scale growth to about one-tenth of the control (Table 4). Therefore, it is evident that scale growth was more severely affected by hypophysectomy than otolith growth. The newly formed region of scales following hypophysectomy was positively stained by sil-

ver nitrate. The marginal osteoid was poorly developed in the scales (Fig. 1). X-ray microanalyses revealed no essential difference in the energy spectra between experimental and control scales. Major elemental constituents in the newly formed region were calcium and phosphorus (Fig. 3), which are probably present in apatite. No spectrum difference was found between the ridge and inter-ridge regions.

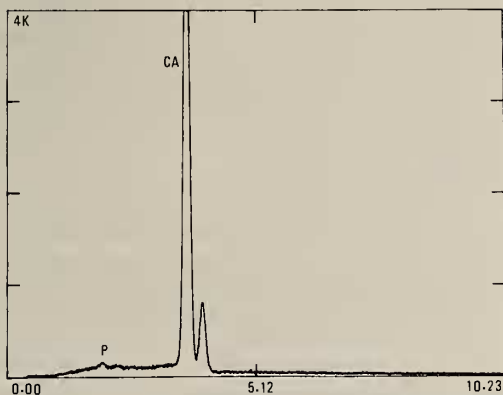


FIG. 2. X-ray energy spectrum from the newly formed region (cf. Fig. 1b) of an otolith after hypophysectomy. The major elemental constituent is Ca.

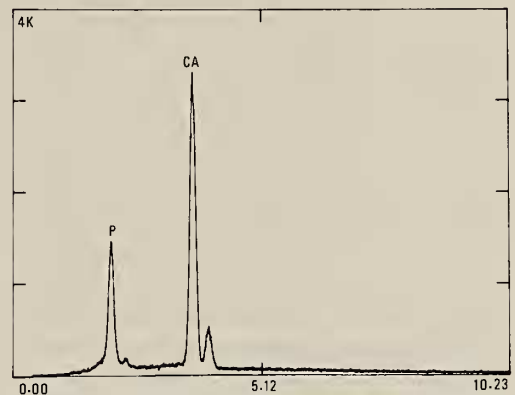


FIG. 3. X-ray energy spectrum from the newly formed region (cf. Fig. 1d) of a scale after hypophysectomy. The major elemental constituents are Ca and P.

DISCUSSION

The pituitary secretes various kinds of hor-

FIG. 1. Otoliths (asterisci) and scales of goldfish at the dorsal and anterior margins, respectively. Arrows represent tetracycline-induced fluorescent bands. White asterisks indicate the spots when X-ray microanalyses were performed (Figs. 2 and 3). Scale bars are 0.1 mm. (a) and (b) Fluorescent images of vertically ground otoliths from sham-operated (a) and hypophysectomized (b) fish. (c) and (d) Fluorescent images of scales from sham-operated (c) and hypophysectomized (d) fish. (e) and (f) Scales stained with silver nitrate. The osteoid (o) is well developed in sham-operated fish (e), but hypophysectomized fish (f) lack the osteoid.

mones. Therefore, the removal of the gland severely affects the fish: cessation in somatic growth [2], disturbances in ion and water balance [8], and arrest in sexual development [9]. The present results of the removal of the pituitary induced cessation in somatic growth in the length of goldfish, which agrees with earlier reports in other fish species [2, 4]. Scale growth was markedly inhibited by hypophysectomy, while otoliths continued to grow at a reduced rate after hypophysectomy, showing an allometric relationship to somatic growth. Outgrowth and calcification in scales are preceded by the formation of marginal osteoid [10], which is collagenous in nature. In hypophysectomized fish, little or no osteoid was found at the scale margin. Therefore, hypophysectomy appears to affect scale growth by retarding osteoid formation. Retardation in osteoid formation probably resulted from the lack of somatotropin, which stimulates the synthesis of proteins and other matrix-related macromolecules via somatomedin [11]. Replacement therapy with somatotropin was effective in restoring hypophysectomy-induced cessation in somatic growth in killifish [3] and bullhead [12].

The above-mentioned interpretation is fundamentally applicable to the effect on otolith growth. However, since otoliths are a highly calcified tissue and the amount of organic matrix is much less than that of scales [13], the relative contribution of the matrix to accretive growth may be not so critical as in scales. According to Degens *et al.* [14], otoliths grow by two processes: the new deposition of calcium as carbonate on non-collagenous matrix and successive growth of the crystals by epitaxy. The latter is a physicochemical process and therefore may be less affected by hypophysectomy. Even if the former phase of matrix formation is reduced to some extent, otoliths could continue to grow at a reduced rate through the process of crystal growth, because the endolymph bathing otoliths are considered to be highly supersaturated to the otolith mineral, calcium carbonate, in goldfish [15].

Allometric growth of otoliths to somatic growth has been reported especially in suboptimal environmental conditions such as low temperatures and food deprivation [16, 17]. Secor and Dean [1]

were the first to explain the allometry by daily increment formation in otoliths. They deduced that a range of somatic growth effects on otolith allometry can be explained by two associated processes: incremental growth of otoliths during periods of arrested somatic growth and continuous growth in some proportion to somatic growth. They have proposed to call such otolith growth pattern the daily increment packing model. Although the DIP model cannot be directly applied to the hypophysectomy-induced asynchronous relationship, it will be necessary to examine incremental microstructures in the newly formed region of otoliths in hypophysectomized fish.

Hypophysectomy induced hypocalcemia in killifish [18, 19] and goldfish [5]. One possible cause for this is a reduction in branchial uptake of calcium [5]. However, hypercalcemia was the case with the present study which focused on long-term effects of hypophysectomy. Since the experimental fish showed no gain in length during the experiment, an increase in bone mass is not expected. Since most calcium (more than 80%) taken up by goldfish is distributed in bone and scales [13], cessation in new skeletal formation would result in an excess of plasma calcium even at a reduced rate of branchial calcium uptake. Therefore, the present increase in plasma calcium concentrations following hypophysectomy can be attributed to the secondary effect of reduced skeletal tissue growth.

Then, why did hypophysectomy induce hypocalcemia and hypercalcemia? Mugiya and Odawara [5] showed that calcium incorporation into scales was not affected by hypophysectomy 2 weeks after operation. This is supported by the present result that 1 or 2 ridges were newly added to scales after hypophysectomy. Therefore, it is reasonable to suppose that inhibitory effects of hypophysectomy on skeletal tissue growth become effective with a lagged period of a few weeks after operation. This would partially explain the conflicting effects of hypophysectomy on plasma calcium concentrations in goldfish in the short-term and long-term experiments.

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